Art. XV.—*Experiments on the Range of Action of the Digestive Ferments.*

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[Read November 5, 1887.]

No subject in the whole range of physiology has had more attention given to it, than that of digestion. Especially since Dr. Beaumont published the results of his observations and experiments on St. Martin, there has been almost an uninterrupted series of investigations into the properties of the digestive juices, and the ferments contained in them. But in spite of the excellent work done, there are still points left unsettled, this being true especially of the active constituents of the pancreatic juice. It has long been known that the pancreas forms a secretion possessed of very powerful digestive properties, and these of a very mixed kind. It has been proved to be capable of digesting all three of the chief ingredients of food, viz., the albumens, the fats, and starch, though there has not been much progress made in the direction of isolating, in a pure state, the ferments which exert these actions. Pancreatine, or pancreatic extract, is assumed to contain at least three distinct ferments—*trypsin*, the solvent of albuminous substances; *steatopsin*, that which emulsifies fats, and splits them up into their constituents; and *amylopsin*, the ferment which converts starch into sugar. Of these, only the first has been obtained in the separate state, and in a tolerably pure form; but of them all it is known, that they exert their special actions best, if not only, in alkaline or neutral media. The pancreatic juice itself is alkaline in reaction, and complete neutralisation of the acid contents of the stomach, when poured into the small intestine, is secured by the further help of the bile which is also strongly alkaline. But, while it has been sufficiently shown that the pancreatic secretion, in the fresh state or in the form of an extract, does convert starch into sugar, and albumen into peptones, in an alkaline mixture, there has been almost no exact enquiry into the influence exerted on it by acidulation of the media in which it may be called upon to act. And

yet this is by no means an idle enquiry. For although within the body, under normal conditions, an alkaline, or at least a neutral reaction of the chyme may be secured, almost immediately after it has passed out of the stomach, there is sufficient practical reason for desiring to know whether the activity of the pancreatic ferments is stopped by the presence of an acid, and if so, in what degree of concentration. For these ferments have now entered largely into commerce, and are used in various ways as helps to digestion. It is important to know the limits to the range of their action, so that agents which are powerful for good, when rightly used, may not be misapplied. And further, it is interesting to know the fate of such agents, when subjected to conditions other than those they ordinarily meet with; whether, that is to say, their powers are only kept in abeyance temporarily, or are completely destroyed.

Experiments with the view of testing these points have become possible, only since good and reliable forms of these ferments have been prepared, and the importance of having them tested is increased by the fact, that they are now largely used in practical medicine. The first of these uses is in the preparation of artificially digested food, for administration in cases where digestion is greatly impaired, or where for any reason it is desired to spare the labour to the stomach involved in carrying on the process of digestion. For this purpose, the pancreatic ferments have a marked superiority over pepsin, which acts only on albumens, and does so only in acid solutions. But when it is proposed further to give these pancreatic preparations internally, as a help to digestion, the question is at once raised, whether there is not simple waste in doing so, there being considerable grounds for supposing that their powers are in complete abeyance in the presence of the acid of the gastric juice. And even supposing that this abeyance of activity is proved to come about in the stomach, there remains the further question, whether the ferments themselves are actually destroyed by continued exposure to the action of the acid and pepsin of the gastric juice, or are capable of resuming activity when an alkaline reaction is again brought about in the duodenum. It was for the purpose of testing these points that the following experiments were devised. The preparation tested was the article of well-established activity, known as zymine, a powder containing the mixed ferments formed by the pancreas. In the stomach

there may be found a variable amount of acid *(hydrochloric acid)*, according to the stage and activity of digestion, and the character of the food; but there is good reason to suppose that it readily reaches the proportion of 1 part in 500 of the mixed contents of the stomach. A considerable time, as much as three or four hours, may elapse before the extreme degree of acidity is reached; but there can be no doubt, after the observations of Beaumont and others, that an intensely acid secretion is poured out as soon as the lining membrane of the stomach is stimulated by the contact of food.

The first series of experiments consisted in heating a mucilage of 10 grains of arrowroot in 20 cubic centimetres of water, for two hours, at 95° F., under different conditions as regards reaction and presence or absence of the ferment :—

I. Mucilage heated alone for two hours, still remained thick, and would not filter.

II. Mucilage heated, as above, with addition of 2 grains each of zymine and bicarbonate of soda. In a few minutes there was distinct thinning of the mixture, which at the end of the time was quite liquid, and filtered easily.

III. Mucilage, with 2 grains of zymine only. The result was the same as in No. II., though the mixture had a very slight acid reaction.

IV. Mucilage, with $\frac{1}{10000}$ part of hydrochloric acid, and 2 grains of zymine.

V. The same, but $\frac{1}{5000}$ part of acid.

VI. The same, but $\frac{1}{1000}$ part of acid.

Even with No. IV., there was some retardation of the solvent action, while with No. V., and still more with No. VI., there was a considerable amount of the swollen starch left in clotty pieces at the end of two hours.

VII. and VIII. To test this effect further, and with reference both to the amylolytic and tryptic elements in the mixed ferment, 10 grains of zymine were heated for two hours at 95° F., in 40 c. ctrs. of water, containing $\frac{1}{1000}$ part of hydrochloric acid. The mixture was then divided into two equal parts, to one of which was added pressed fibrin, 10 grains, and to the other, 10 grains of starch boiled in 20 c. cs. of water. Each of these was again kept for a full

hour at 95° F., but little if any solvent action was observed at the end of that time.

It was thus made clear that the action of the mixed ferment is almost completely checked in the presence of hydrochloric acid, in the proportion of 1 part per 1000, and to a considerable extent when the acid was present in the strength of 1 to 5000.

IX. For the purpose of discovering whether loss of power was only temporary, or if the ferment had been permanently injured; the mixtures of starch and acid (Exps. IV., V., and VI.) were rendered alkaline, by the addition of 2 grains of bicarbonate of soda to each, and again kept at 95° F. for two hours. In that which had contained only $\frac{1}{10000}$ of the acid, there was complete liquefaction, while in those which had contained $\frac{1}{5000}$ and $\frac{1}{1000}$ respectively, there was slight breaking down of the clotty particles, but no great change. In both of these, therefore, there had been permanent injury to the ferment. though it did not seem to be completely destroyed.

X. For the purpose of discovering whether this destruction would be effected by the presence of pepsin in the acid mixture, the following experiment was carried out. Zymine (10 grains) was heated for two hours at 95° F., in 40 c. ctrs. of water, with 10 grains of Fairchild's scale pepsin, acidulated to the strength of 1 in 1000. At the end of that time the mixture was divided into two equal parts, each of which was rendered slightly alkaline with bicarbonate of soda, and heated again for an hour with 10 grains of pressed fibrin, and the mucilage of 10 grains of starch respectively. It was found that both the fibrin and the starch were almost completely dissolved. This difference from the experiment before detailed (IX.), was due probably to the larger amount of zymine present, 5 grains instead of 2. It was made clear that the pepsin, as such, had not acted at all on the zymine, though placed under very favourable conditions for doing so.

For the sake of completeness, the following counter-experiment was tried:—

XI. Five grains of pepsin were kept at 95° F. for 2 hours, along with 5 grains of zymine and 2 grains of bicarbonate of soda. Hydrochloric acid was then added in sufficient amount

not only to neutralise the soda, but to leave an excess equal to 1 part in 500 of the mixture. To this was then added coagulated white of egg in thin slices, and the whole kept for 3 hours at 100° F. At the end of that time the albumen was not appreciably altered ; while similar slices, treated in the same way with fresh pepsin and hydrochloric acid, 1 in 500, were found, at the end of 3 hours, to be completely dissolved. The quality of the pepsin being thus shown to be good, it follows that the treatment to which it had been subjected had had a destructive influence on it. Whether this was owing chiefly to the action of the bicarbonate of soda, or to that of the zymine, remains, of course, undetermined, though the probability is that the latter supposition is the correct one.

As this question did not enter into the scope of the original inquiry, though it is of great interest, it had to be left, the investigation having proved sufficiently laborious.

The general conclusions are :—

I. That the pancreatic ferments are not merely temporarily inhibited in their digestive action by small quantities of hydrochloric acid, but are permanently injured, when the strength of the acid reaches the proportion of 1 to 1000, or even 1 to 5000, for 2 hours.

II. That pepsin does not seem to have any power, in association with the acid, in bringing about or even hastening this destructive action.

III. That, on the contrary, the trypsin of the pancreatic secretion seems to bring about the destruction of pepsin in slightly alkaline solutions.

I have to acknowledge my great obligations to Mr. Frederick Dunn, public analyst, for assistance in the way of carrying out the practical details of the experiments. Without that assistance, indeed, I fear that the inquiry would scarcely have been carried out at all.